PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :		(11) International Publication Number:
A61K 38/03, C07K 14/705	A1	WO 98/15282
		(43) International Publication Date: 16 April 1998 (16.04.98)

(21) International Application Number: PCT/IT97/00240

(22) International Filing Date: 9 October 1997 (09.10.97)

(30) Priority Data:
RM96A000687 9 October 1996 (09.10.96) IT

(71) Applicant *(for all designated States except US)*: ISTITUTO FARMACOTERAPICO ITALIANO S.P.A. [IT/IT]; Via Paolo Frisi, 21/23, I–00197 Roma (IT).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: TARRO, Giulio [IT/IT]; Istituto Farmacoterapico Italiano S.p.A., Via Paolo Frisi, 21/23, I–00197 Roma (IT).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: IMMUNOGENIC TLP COMPOSITION

(57) Abstract

The present invention relates to an immunogenic composition comprising at least one protein from TLP or a fragment thereof, and in particular to the compositions wherein said fragments can comprise at least one of the peptides claimed as SEQ ID NO. 1, SEQ ID NO. 2 and SEQ ID NO. 3 in the European Patent No. 9391641.0, or the peptide claimed as SEQ ID NO. 1 in the Italian Patent Application No. RM96A000496, suitable in therapy against tumoral diseases, and in particular against NSCLC and uro–genital cancer.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

IMMUNOGENIC TLP COMPOSITION

DESCRIPTION

Background of the invention

The present invention relates to the field of immunotherapy of tumoral diseases.

Prior art

The oncologic research has been directing its efforts for many times towards the problem of immunotherapy of tumoral diseases, on the basis of the reasonable possibility of finding a therapeutically useful solution through the manipulation of the immuno-oncolytic reaction that the human organism can develop spontaneously. The initial urge towards such trend of the scientific research can be recognised in the first observations (I.S. Irlin, Virology 1967 32:725; E.Klen et al. Nat.Cancer Inst.1964 32:547; G.J.Pasternak J.Nat.Cancer Inst., 1965, 34:71, S.S. Tevethia et al. Immunol. 1968, 100:358; R.Nishioka et al. Monograph 1968, 7:49)regarding the stimulation of specific humoral and cellular antibodies by the antigens of neoplastic cells both in animals and in human beings.

The immune manipulations attempted so far as an immunotherapic approach, while reflecting the knowledge successively acquired concerning the physiopathology of cancer and of the immune system of the host, on the other side also suffers from the lack of suitable immunogenic agents or from the difficulty experienced in removing the situations of block of cellular immunity which are present in the cancer patients.

As a matter of practice two main roads are originally followed:

a) the non specific activation of the host's immunity in order to strengthen the immuno-oncolytic reactions (immuno-adjuvants);

b) the specific activation of the host's immunity in order to electively stimulate the production of antibodies having oncolytic effects.

The specific approach has the advantage to direct a more specific and effective immune-response to the tumor cells, strenghthening the effects.

In any case, the lysis of tumour cells by the immune system is mediated both by direct cytotoxic mechanisms (NK cells) and by cytotoxic mechanisms that are more complex but more effective and specific, involving recognition of antigens on the surface of the tumour cells and the presence of antibodies (ADCC cytotoxicity, prevalently mediated by CD8+ cells). The immune system always operates by means of a complex network of cytokines, which modulate the action of the effector cells by means of inhibition and stimulation, with a "cascade" mechanism.

Attempts in immunotherapy have up to date been aimed at generic amplification of the cell-mediator immune response, either expanding (thymic hormones, IL-2) or activating (BCG, PPD, IFNs, IL-2, TNFs, etc.) the lymphocyte populations in a non-specific manner. Research into this effect was also through the use of a single substance (IL-2, IFN, etc.) which in order to induce the necessary function required to be administered at high doses, with considerable toxic effects and prohibitively high costs (Mulè JJ, Shu S., Swarz SL., Rosenberg SA., Science, 225: 1478, 1984; Hadden JW., in "Advances inimmunomodulation"., 1988, Ed. B. Bizzini and E. Bonmassar, Pitagora Press Roma).

Previous studies have shown us that combined immunotherapy (thymic hormones and a cytokine administered at a low dose, IFN-alpha or IL-2) was capable of producing a synergetic effect on the cytotoxic activities of the lymphocytes (NK, LAK, CTL activity) with respect to certain tumour cells *in vitro* (Favalli, C., Mastino, A., Jezzi, T., Grelli, S., Goldestein, A.L. and Garaci, E., Int. J. Immunopharmacol., 11, 443-450, 1989; Mastino, A., Favalli, C., Grelli, S., Innocenti, F. and Garaci, E., Cell. Immunol., 133, 196-205, 1991).

However, an increase in cytotoxic activities corresponded neither to an adequate anti-tumour response *in vivo*, nor to an increased survival rate (Favalli, C., Mastino, A., Grelli, S., Pica, F., Rasi, G., Garaci, E., Combination Therapies. pp. 275-280 Ed. by A. Goldstein and E. Garaci, Plenum Press, NY, 1992; Mastino, A., Favalli, C., Grelli, S., Rasi, G., Pica, F., Goldestein, A.L. and Garaci, E., Int. J. Cancer. 50, 493-499, 1992; Garaci, E., Pica, F., Mastino, A., Palamara, A.T., Belardelli, F. and Favalli, C., J. Immunother., 13, 7-17, 1993). On the contrary, when combined immunotherapy was preceded by chemotherapy (even using ineffective doses) there was on the other hand complete recovery from the tumour (Mastino, A., Favalli, C., Grelli, S., Rasi, G., Pica, F., Goldestein, A.L. and Garaci, E., Int. J. Cancer. 50, 493-499, 1992; Garaci, E., Pica, F., Mastino, A., Palamara, A.T., Belardelli, F. and Favalli, C., J. Immunother., 13, 7-17, 1993; Rasi, G., Sinibaldi-Vallebona P., Favalli, C., Pierimarchi, P., et al., 2nd International Symposium on Combination Therapies, documents, 1-3 May, 1992 - Santa Tecla CT).

Further studies on experimental models have shown that the immunotherapy was only effective in case of immunogenic neoplasia, and that the main role of chemotherapy (used at ineffective doses) was to render the neoplasia immunogenic (Rasi, G., Sinibaldi-Vallebona P., Favalli, C., Pierimarchi, P., et al., 2nd International Symposium on Combination Therapies, documents, 1-3 May, 1992 - Santa Tecla CT; Sinibaldi-Vallebona P., Pierimarchi, P., Ravagnan, G.P., Rasi, G., Third International Symposium on Combination Therapies, documents, 29-31 October, 1993, Houston, Texas; Sinibaldi-Vallebona P., Pierimarchi, P., Lucertini, L., Ravagnan, G.P., Rasi, G., Fourth International Symposium on Combination Therapies, 14-17 June, 1994, documents, p. 105; Rasi, G., Silecchia, G.F., Sinibaldi-Vallebona P., Pierimarchi, P., Sivilia, M., Tremiterra, S., Garaci, E.,

Int. J. Cancer, 57, 701-705, 1994). Recently, this strategy has also been found to be effective in treatment of solid human tumours (Rasi, G., Favalli, C., Terzoli, E., Izzo, F., Sinibaldi-Vallebona P., Pierimarchi, P., Sivilia, M., Garaci, E., Biomedicine & pharmacotherapy, 47-292, 1993). In experimental models we have thus demonstrated the low effect or lack of effect of each of the single treatments (chemotherapy, thymic hormone, cytokine), the absence of anti-tumour effect even in the presence of a considerable increase in the levels of immune activity in cells, and lysis of the tumour only in the presence of a specific cell-modulated cytotoxic activity on neoplastic cells. From these studies it can therefore be stated that the role of the antigen is decisive in order to induce a cytotoxic immune response with a significant anti-tumour effect.

When attempting to amplify the anti-neoplastic immune response the availability of antigens to induce and modulate, and the knowledge of the immunological relationships within each "target/effector" system (neoplastic cell/lymphocyte) therefore appear to be essential.

TLP complexes are protein complexes present in human tumour cells. Among these TLP proteins a protein of 240 Kda is described (Tarro G., Oncology 40, 248-253, 1983). TLP are isolated from tumour tissues as described in the European patent No. 0283443. The European patent No. 649433 identifies a TLP protein obtained from pulmonary carcinoma. The Italian patent application No. RM96A000496 indicates that TLP obtained from carcinomas of the uro-genital system comprise peptised of a different sequence than those previously identified. The proteic fragments of proteins from TLP can also be produced synthetically using known methods.

In 1983 a new tumour antigen of 240 kDa was identified, extracted from the neoplastic tissue of non-small cell lung carcinoma (NSCLC) and named TLP (Tumour

Liberated Particles). In the Cold Spring Harbor Laboratories (NY-USA) a structure analyisis of the lung carcinoma antigen was recently performed and 100 kDa antigen was extracted, then a major TLP epitope sequence was recently identified, the polypeptide (a nonapeptide, CSH 275) was synthesised, and the relative antibody (CSH 419) was produced.

This antibody (CSH419) has proved its ability, using Western blot after immunoprecipitation and immunohystochemistry (P.A.P.), to recognise the antigen sequence in the homogenate obtained from all the neoplastic tissues (NSCLC) taken into consideration up to this point.

The peptide claimed in Seq. ID N1, with others derived from the antigenic region of TLP were described and claimed in the European Patent no. 649433 corresponding to the International Application WO-A-001458.

SUMMARY OF THE INVENTION

The present invention relates to a pharmaceutical composition containing at least one protein of a TLP (Tumour Liberated Particles) complex for therapeutic and immunogenic use.

The author of the present invention has now prepared full and accurate documentation of clinical use of TLP as an immuno-modulating agent (capable of stimulating the immune responses of the host) both to combat diagnosed neoplastic pathologies (immuno-therapy), and to prevent cancerous pathologies (vaccine).

On the basis of experience gained during study of experimental models, the essential requirements to enable an antigen like TLP to be used from a therapeutic point of view are the following:

1. the presence of the antigen on the surface of the tumour cells;

2. the ability to pharmacologically induce or increase expression of the antigen on the surface of the cells;

3. the ability of the antigen to stimulate lymphocytes with a specific anti-tumour activity (blastogenetic capacity and CTL (cytotoxic tymus dependent lymphocytes) activity);

4. the presence of TLP in the serum (indicates possible correlation between the serum levels and expression of the antigen on the cell surface, and the absence of systems (kidneys or other emunctory systems such as the skin or intestine) giving rapid clearance from circulation).

The positivity to these four operations are indicative of both a suitability as a therapeutic agent and as a vaccine, owing to the strong immunogenic activity demonstrated.

The object of the present invention is therefore an immunogenic composition and the use therof as a vaccine and as a medicament in the prevention and the treatment respectively of cancer, particularly pulmonary cancer and uro-genital cancer, comprising at least one protein obtained from TLP or at least an immunogenic fragment thereof.

The immunogenic phragments of TLP protein preferably contain at least one of the following amino acid sequences:

ArgThrAsnLysGluAlaSerIle (Seq ID N1 of WO-A-001458)

GlySerAlaXPheThrAsn (Seq ID N2 of WO-A-001458)

AsnGlnArgAsnArgAsp (Seq ID N3 of WO-A-001458)

Alternatively, the immunogenic composition according to the invention includes an immunogenic fragment comprising the following amino acid sequence:

GlyProProGluValGlnAsnAlaAsn (Seq ID N1 of Italian patent application RM96A000496).

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the result of an experiment carried out on neoplastic cells obtained from NSCLC explants using the flow cytofluorimetry technique (Facscan-BD).

The peak on the right of the histogram, reveals the binding of the anti-TLP antibodies iwth the correspondent antigen on the cell surface.

Figure 2 shows the result of the same experiment described in figure 1, carried out on cells from the same explants, treated with pre-immune serum as a negative control.

The peak showed in figure 1, comprising the values between 10^3 and 10^4 of the fluorescence, and revealing the bond between antigen and antibodies anti-TLP, is absent.

EXPERIMENTAL DESCRIPTION

Presence of the antigen TLP on the surface of the tumour cells.

The antigen TLP was searched for on the surface of fresh neoplastic cells obtained from non-small cell lung carcinoma explants (NSCLC) using the flow cytofluorimetry technique (Facscan-BD).

The cells were marked by addition of monoclonal anti-TLP antibody (CSH # 419, Cold Spring Harbor Lab. NJ USA) in conjunction (second step) with a second goat anti-rabbit IgG-RPE.

The TLP-antiTLP binding specificity was evaluated using non-specific antiserums or pre-immune serum. The cell phenotype specificity was evaluated by marking cells of stabilised tumour lines or fresh cells from neoplasias other than NSCLC with antiTLP.

Ability to pharmaceutically induce or increase expression of the antigen on the surface of the cells.

The cells were processed fresh and after preparation of primary cultures (complete RPMI 1640 culture medium with the addition of FCS 10%).

Along with variation in TLP, other possible phenotype modifications (IL-2 rec (interleukin

receptors), HLA-Dr (human leukocyte antigen), CD16 (lymphocyte sub-populations), etc.) induced by treatment with single or combined agents (chemotherapy, cytokines) were also studied.

In the case of TLP, freshly isolated tumor cells were obtained from surgical NSCLC patient; after 24 plating and fibroblasts separation, cells were resuspended for immunoistochemistry and cytofluorimetric assay, briefly: CSH419 antiserum was labeled with PE coniugated anti-rabbit IgG and incubated with the cells. Negative controls were obtained by the use of rabbit pre-immune serum and (for immunoistochemistry also by serial dilution down to 500 fold for positive samples; no reactions was observed by staining or conjugating K562, and 2 women melanoma cell line. TLP was demonstrated on 75% of the NCLC lines studied.

Preliminary studies by confocal microscopy showed a cytoplasmatic and membrane localization of TLP by the tumor cells. TLP antigen expression has been shown to be enhanced or induced in vitro by chemotherapy treatment: primary NSCLC culture cell become TLP-positive after cisplatinum or etoposide treatment.

The freeing of TLP in the culture supernatant was checked using the ELISA test.

Simultaneously experiment consistent in administration of cis-platinum and etoposide to serum-negative NSCLC patients are carried out to test the reaction on TP production in vivo.

Ability of Ag to stimulate lymphocytes with specific anti-tumour activity (blastogenetic ability and CTL activity).

The lymphocytes obtained from peripheral bloodstream of patients from whom the neoplastic cells were explanted (autologous lymphocytes) were marked by flow cytofluorimetry (CD4/CD25, CD8/CD25, CD56-16-3±/CD25 phenotypes) before and after treatment in vitro with TLP,

both alone and in association with chemotherapy and/or cytokines.

The cytotoxic activity of the autologous lymphocytes (both treated and untreated) was determined by testing release of the ^{51}CR after 4h, using the tumour cells obtained from explants of tumour from the same patient as target cells.

Lymphocytes from healthy individuals and from patients suffering from other neoplastic pathologies were used (as effectors) against neoplastic cell lines (sensitive and resistant NK targets) as controls to establish the specific nature of the tumour lysis.

Presence of TLP in the serum

A test ELISA with analytical "sandwich" scheme, was carried out to determine the presence of TLP and in the serum of NSCLC patients, and in serum of patient affected by other pathologies (neoplastic pathologies different from NSCLC; lung non-neoplastic pathologies) and in other controls.

RESULTS

Presence of the antigen TLP on the surface of the tumour cells.

The presence of TLP on lung carcinoma cells (NSCLC) was demonstrated using the method described. As an example of positive tumour, the data shown in figure 1 are reported. It is possible to note that the labeling with pre-immune serum gives no signal, while the labeling with antiTLP#419, distinctly evidences a TLP positive population.

Ability to pharmaceutically induce or increase expression of the antigen on the surface of the cells.

Two TLP-positive tumor cell population were treated with cisplatinum and etoposide (10µg/ml) for 48 hours:

2 TLP-negative cell population became TLP-positive after etopside treatment;

1 TLP-negative cell population became TLP-positive after cisplatinum treatment;

TLP positive cell population remained TLP positive after etoposide and cisplatinum treatment.

The results of the ELISA test used for checking the freeing of TLP in the culture supernatant are compatible to that exposed in tables II-IV, reporting the presence of TLP in the serum of NSCLC patients.

The administration of the same chemotherapics in TLP serum-negative patients, has produced an intensive positive response for TLP, checked by ELISA, after only two cycles of treatment.

Ability of Ag to stimulate lymphocytes with specific anti-tumour activity (blastogenetic ability and CTL activity).

In vitro treatment of the lymphocytes of patients suffering from NSCLC with TLP induces blastic activity, in particular against certain phenotypes: activated cells that express the high affinity receptor for IL-2 (CD3+/CD25+); NK cells (CD56+/CD16+/CD3±); activated cytotoxic cells CD25+/CD8+.

Table I

PHENOTYPES	CD25	NK	CD25/CD8
Untreated lymphocytes	3.3-4.6	10.1-18.9	3.5-5.3
Lymphocytes + TPL*	5.5-16.1	21.4-32.2	8.6-11.3**

* µg/ml

** a dose-dependent effect is observed for addition of TLP to the medium and activation of the CD8+ cells (which can reach up to 20% of the entire culture population).

Treatment of the lymphocytes with TLP is also capable of inducing lytic activity of both type NK (natural killer cells, target cells K562) and CTL (on cells of its own tumour). The NK activity shows the same dose dependence seen for CD8+CD25+ cells and also appears to be closely correlated to the number of said cells. This observation, along with the absence of lytic activity (either spontaneous or TLP-induced) of a LAK type (lymphokine activated killer cells, targeting NK-

resistant cells, Daudi) appears to indicate the specific nature of the activation.

The homologated lymphocytes, treated with TLP, have also shown themselves to be active on cells of a cerebral metastasis resulting from NSCLC.

Presence of TLP in the serum

The results of the ELISA with analytical "sandwich" scheme has given the resulte exposed as follows:

Table II

NSCLC

HYSTOLOGIC TYPE	N/POS.	%
Epidermoidal	40/22	55
Adenocarcinoma	12/7	58
Tot.	**52/29**	**56**

Table III

Different neoplasias from NSCLC

HYSTOLOGICAL TYPE	NEG.	%
SCLC	15/15	100
Indefinite	7/7	100
Carcinosarcoma	1/1	100
Carcinoid	1/1	100
Pulm. Metast. from ovary	1/0*	
Melanoma	3/3	100
Gastric Carcinoma	2/2	100
Tot.	**30/29**	**97**

* Border line

Table IV

Non-neoplasia lung pathologies

PATHOLOGY	N/NEG.	%
BOC	21/18	86
TBC	2/1	50
Tot.	**23/19**	**82**

Table V

Other controls

Cases	N/Neg.	%

Healthy	11/11	100
Pregnant	2/1	50
Tot.	**13/12**	**92**

Moreover, the measurement of the TLP levels in NSCLC patients gives a results of 56% (see table II) which must be compared with the data of 35-40% obtained from the "cyfra", the other lonely marker proposed for Lung carcinoma. Furthermore the data on the specificity of the binding indicate a specificity of 100% circa, for the TLP against a datum of 60%-70% for the "cyfra".

In a similar manner, the synthetic polypeptide (Seq. ID N1 of W-A-001458) shows excellent immunogenic abilities, studies actually indicating a specific stimulation of the cytotoxic CD8 lymphocytes only in patents suffering from NSCLS neoplasia.

Esperiments carried out in "skid mice" show that a vaccinal tratment with Seq. ID N1 of W-A-001458 protects the animals from a growth of 1.800.000 to 6.000.000 of tumour cells.

Analogous results are obtained for the other peptides claimed.

FINAL CONCLUSION

The results of the studies carried out on TLP, the derivative peptides, and the peptide GlyProProGluValGlnAsnAlaAsn derived from the analogous protein extracted from urogenital-carcinoma, show an immunological anti-tumoral action directed against them.

In fact the presence of TLP on the surface of the NSCLC cells, and its demonstrated capability to stimulate lymphocytes with specific anti-tumour activity (both the blastogenetic ability and CTL activity), evidence clearly the immunogenicity of the protein. The same considerations are demonstrated to have value for the peptides derived, both Seq. ID N1 of W-A-001458 and the other two peptides Seq. ID N2 and Seq. ID N3 also derived from the antigenic region of the TLP protein.

The value of this approach is confirmed by the fact that a relevant presence of TLP in the serum of NSCLC patients was found, indicating a strict association between the presence of tumour and the level of TLP in blood. Therefore it could be suitable and effective an approach based on the administration of TLP, or the peptides thereof, as its immunogenically active sequence were demonstrated to have no homologous in other human protein.

The administration of TLP or derived peptides therefore, would be more specific and effective than an approach carried out by a non-specific activation of the host's immunity system, and less destructive than the administration of the solely chemotherapics.

Howewer the administratiom of the chemotherapics could also potentiate the therapeutic effects of TLP, as they have demonstrated the capability of inducing the production of the Ag both in cell cultures and in NSCLC patients originally serum-negative for TLP.

This is a direct consequence of the results of the experiment carried out with single or combined chemiotherapic agents. As it was previously shown in fact, the administration of etoposide and cis-platinum in tumour cell cultures was shown to stimulate TLP production at levels compatible with those registered in the NSCLC serum-positive patients, and the administration of the same chemotherapics make the patients originally serum-negative (ELISA) for TLP, to become intensively positive after two cycles of chemotherapy.

In the case of the peptide GlyProProGluValGlnAsnAlaAsn derived from the protein analogous to TLP, extracted from urogenital-carcinoma the experiment carried out demonstrate an analogous suitabiity as immunotherapic for all the urogenital tumoral forms.

All these data are indicative of both a therapeutic and a vaccinal use owing to the the strong immungenic

activity demonstrated from all these molecules. This use is suitable for humans and mammals in general for proteins obtained from TLP and analogous thereof. The same is true for the peptides illustrated in the experimental texts.

The immune response in fact can be caused both to contrast the tumour growth and expansion (treatment effective also in metastasis as it is previously shown) and to protect healthy people from developing the disease. The data obtained in "skid mice" previously shown particularly support these statements.

CLAIMS

1. An immunogenic composition comprising at least one protein obtained from TLP or at least one immunogenic fragment thereof, in a pharmaceutically effective and acceptable dose.

2. An immunogenic composition according to claim 1, in which said immunogenic fragment includes at least one of the amino acid sequences selected from the group comprising:

ArgThrAsnLysGluAlaSerIle (Seq ID N1 of WO-A-001458)

GlySerAlaXPheThrAsn (Seq ID N2 of WO-A-001458)

AsnGlnArgAsnArgAsp (Seq ID N3 of WO-A-001458)

3. An immunogenic composition according to claim 1, in which said immunogenic fragment comprises the following amino acid sequence:

GlyProProGluValGlnAsnAlaAsn (Seq ID N1 of Italian patent application RM96A000496).

4. Use of a TLP protein or at least an immunogenic fragment thereof for the manufacture of a vaccine for a preventive treatment of a cancer desease in mammals.

5. Use of a TLP protein or at least an immunogenic fragment thereof for the manufacture of an immunogenic medicament for an active specific immunotherapic treatment of a cancer desease in mammals.

6. Use according to claim 4 or 5 in which said cancer desease is lung cancer.

7. Use according to claim 6 in which said cancer desease is Non-Small Cell Lung Cancer (NSCLC).

8. Use according to claim 4 or 5 in which said cancer desease is uro-genital cancer.

9. Use of an immunogenic fragment of TLP including at least one of the amino acid sequences selected from the group comprising:

ArgThrAsnLysGluAlaSerIle (Seq ID N1 of WO-A-001458)

GlySerAlaXPheThrAsn (Seq ID N2 of WO-A-001458)

AsnGlnArgAsnArgAsp (Seq ID N3 of WO-A-001458)

for the manufacture of a vaccine for a preventive treatment of lung cancer in mammals effective to produce in said mammal an immune response against said lung cancer.

10. Use of an immunogenic fragment of TLP including at least one of the amino acid sequences selected from the group comprising:

ArgThrAsnLysGluAlaSerIle (Seq ID N1 of WO-A-001458)

GlySerAlaXPheThrAsn (Seq ID N2 of WO-A-001458)

AsnGlnArgAsnArgAsp (Seq ID N3 of WO-A-001458) for the manufacture of an immunogenic medicament for an active specific immunotherapic treatment of lung cancer in a mammal, effective to produce in said mammal an active specific immune response against said lung cancer.

11. Use according to claim 9 or 10 in which said lung cancer is Non-Small Cell Lung Cancer (NSCLC).

12. Use of an immunogenic fragment of TLP comprising the following amino acid sequence:

GlyProProGluValGlnAsnAlaAsn (Seq ID N1 of Italian patent application RM96A000496)

for the manufacture of a vaccine for a preventive treatment of uro-genital cancer in mammals.

13. Use of an immunogenic fragment of TLP comprising the following amino acid sequence:

GlyProProGluValGlnAsnAlaAsn (Seq ID N1 of Italian patent application RM96A000496)

for the manufacture of an immunogenic medicament for an active specific immunotherapic treatment of a uro-genital cancer in a mammal effective to produce in said mammal an active specific immune response against said cancer.

14. A method for vaccinating a mammal against a cancer comprising administering to said mammal an amount of a TLP protein or at least one immunogenic fragment

thereof pharmaceutically effective to produce in said mammal an immune response against said cancer.

15. A method for treating a cancer disease in a mammal by an active specific therapy comprising aministering to a mammal in need thereof an amount of a TLP protein or at least one immunogenic fragment thereof pharmaceutically effective to produce in said mammal an active specific immune response against said cancer.

16. A method according to claim 14 or 15 in which said cancer is lung cancer.

17. A method according to claim 16 in which said lung cancer is Non-Small Cell Lung Cancer (NSCLC).

18. A method according to claim 14 or 15 in which said cancer is uro-genital cancer.

19. A method for vaccinating a mammal against lung cancer comprising administering to said mammal an immunogenic fragment of TLP including at least one of the amino acid sequences selected from the group comprising:

ArgThrAsnLysGluAlaSerIle (Seq ID N1 of WO-A-001458)

GlySerAlaXPheThrAsn (Seq ID N2 of WO-A-001458)

AsnGlnArgAsnArgAsp (Seq ID N3 of WO-A-001458),

in an amount pharmaceutically effective to produce in said mammal an immune response against said lung cancer.

20. A method for treating lung cancer in a mammal by an active specific therapy comprising administering to a mammal in need thereof an immunogenic fragment of TLP including at least one of the amino acid sequences selected from the group comprising:

ArgThrAsnLysGluAlaSerIle (Seq ID N1 of WO-A-001458)

GlySerAlaXPheThrAsn (Seq ID N2 of WO-A-001458)

AsnGlnArgAsnArgAsp (Seq ID N3 of WO-A-001458),

in an amount pharmaceutically effective to produce in said mammal an active specific immune response against said lung cancer.

21. A method according to claim 19 or 20 in which said lung cancer is Non-Small Cell Lung Cancer (NSCLC).

22. A method for vaccinating a mammal against uro-genital cancer comprising administering to said mammal an immunogenic fragment of TLP comprising the following amino acid sequence:

GlyProProGluValGlnAsnAlaAsn (Seq ID N1 of Italian patent application RM96A000496)

in an amount effective to produce in said mammal an immune response against said uro-genital cancer.

23. A method for treating a uro-genital cancer in a mammal by an active specific therapy comprising aministering to a mammal in need thereof an immunogenic fragment of TLP comprising the following amino acid sequence:

GlyProProGluValGlnAsnAlaAsn (Seq ID N1 of Italian patent application RM96A000496)

in an amount effective to produce in said mammal an active specific immune response against said uro-genital cancer.

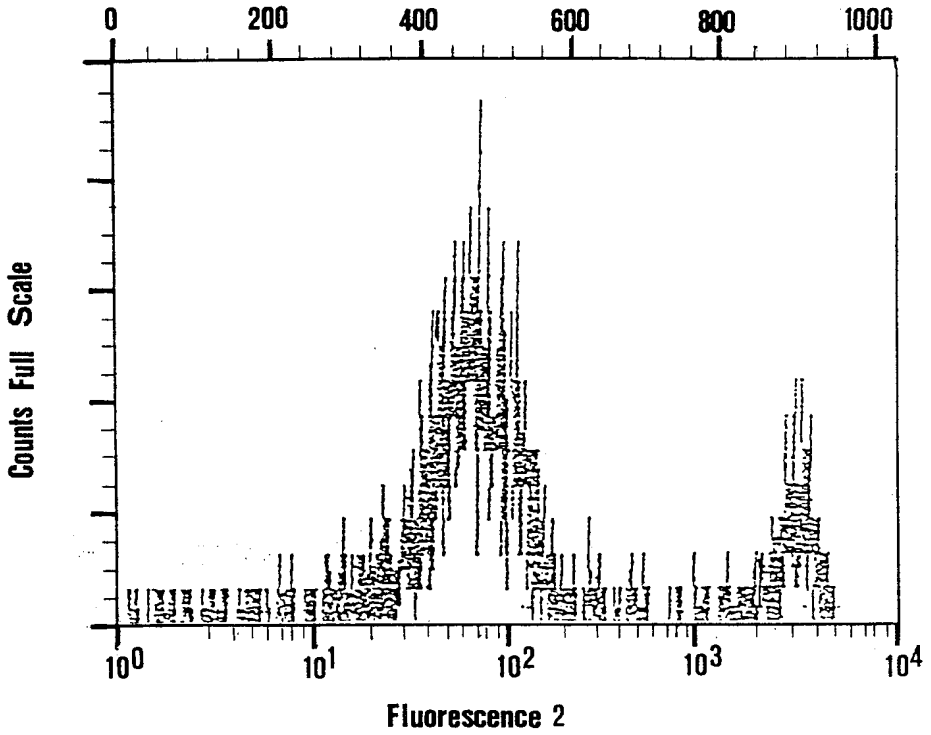
0
200
400
600
800
1000
Counts Full Scale
10^0
10^1
10^2
10^3
10^4
Fluorescence 2

FIG 1

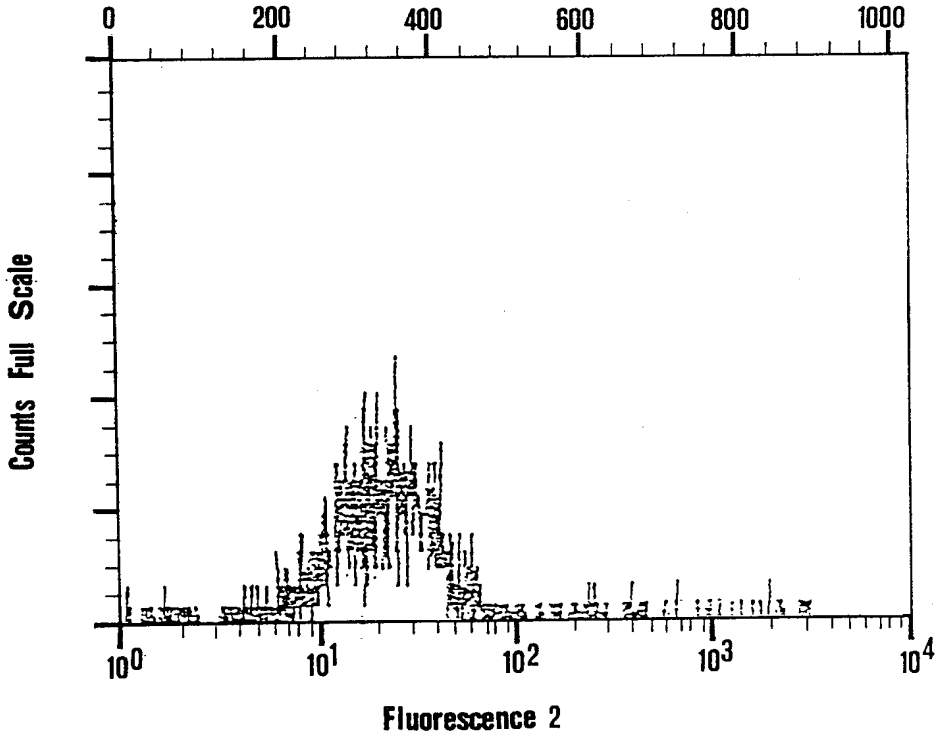
0
200
400
600
800
1000
Counts Full Scale
10^0
10^1
10^2
10^3
10^4
Fluorescence 2

FIG 2

INTERNATIONAL SEARCH REPORT

Int tional Application No
PCT/IT 97/00240

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/03 C07K14/705

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94 01458 A (ISTITUTO FARMACOTERAPICO ITALI ;TARRO GIULIO (IT)) 20 January 1994	1-23
Y	see page 7-8, seq. id. no. 1, 2 and 3	1-23

Y	EP 0 283 443 A (FARMACOTERAPICO IST ITAL) 21 September 1988 see page 5, line 9 - line 14	1-23

A	TARRO, GIULIO ET AL: "Human tumor antigens inducing in vivo delayed hypersensitivity and in vitro mitogenic activity" ONCOLOGY (1983), 40(4), 248-54 CODEN: ONCOBS;ISSN: 0030-2414, XP002045904 see the whole document	

[ ] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
7 January 1998	16. 01. 98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Halle, F

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

....ernational application No.
PCT/IT 97/00240

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

Claims Nos.: 14-23

because they relate to subject matter not required to be searched by this Authority, namely:

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

Remark : Although claims 14-23
are directed to a method of treatment of
the human/animal body , the search has been carried out and based on the
alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/IT 97/00240

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9401458 A	20-01-94	IT 1262954 B	23-07-96
		AT 160359 T	15-12-97
		AU 680198 B	24-07-97
		AU 4582393 A	31-01-94
		CA 2139518 A	20-01-94
		DE 69315341 D	02-01-98
		EP 0649433 A	26-04-95
		JP 7508749 T	28-09-95
EP 0283443 A	21-09-88	DE 3882061 A	05-08-93
		DE 3882061 T	03-02-94
		ES 2061731 T	16-12-94